(basically by comparing the absolute molecular weight with the apparent molecular weight for the linear polymer calibration). The response of the light scattering detector increases dramatically with molecular weight and SEC-light scattering systems are very good at examining any variation at the high molecular weight end of a distribution.

In SEC-light scattering, the solution concentration is an important parameter in the calculation and it is necessary to have accurate information of the differential refractive index for the polymer/solvent (this is a squared term in the calculation). This requirement for information on the differential refractive index is problematic for examination of copolymers.

SEC with Viscosity Measurement

As noted above, it has been empirically demonstrated that for many polymer types, a universal calibration is obtained if the log. product of molecular weight and intrinsic viscosity is used rather than simple log. molecular weight. This is utilized by combining the response of a viscosity detector and a concentration detector to give the universal calibration directly. The viscosity monitor measures the differential pressure as polymer solution travels through a capillary; detectors have been developed which use a single capillary, a pair of capillaries or four capillaries (arranged in a manner analogous to a Wheatstone bridge).

SEC-viscosity is not theoretically an absolute approach but should give the true molecular weight distribution, providing that the polymer of interest conforms to the Universal Calibration approach. As with SEC-light scattering, SEC-viscosity is valuable for obtaining information on branching. Again, the solution concentration is an important parameter in the calculation. The differential refractive index does not appear in the calculation but could produce inaccuracies in the assumed concentration and hence is also problematic for copolymers.

Commercial hardware and software is available for combining SEC-light scattering-viscosity within a single system.

Future Prospects

Although there have been suggestions that other techniques (e.g. matrix-assisted laser-desorption ionization – time-of-flight, MALDI-TOF, mass spectroscopy) might replace SEC, there seems little prospect of this in the near future.

There are new commercial integral SEC systems now available that should simplify some of the more difficult applications and make SEC combined techniques more routine. These developments should ensure that SEC is a main stream technique for the foreseeable future. There will also probably be more utilization of triple-detection (concentration, viscosity and light-scattering) for detailed characterization of specific polymer types.

See also: **II/Chromatography:** Detectors: Laser Light Scattering. **Chromatography: Liquid:** Detectors: Refractive Index Detectors; Theory of Liquid Chromatography. **III/Gradient Polymer Chromatography: Liquid Chromatography. Synthetic Polymers:** Liquid Chromatography.

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Micellar Liquid Chromatography

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Introduction

Surfactants are molecules that exist as monomers when they are at low concentrations in solution, while above their critical micelle concentration (c.m.c.) they associate to form aggregates called micelles. Two zones of different polarity exist in the molecules of surfactants: one is hydrophobic in nature, formed from one or more hydrocarbon chains; the other can be polar or even ionic. According to the nature of this second zone, surfactants are classified into three main categories: ionic (anionic and cationic), nonionic and zwitterionic (amphoteric).

The combination of hydrophobic and hydrophilic properties confers some special characteristics on micellar systems in aqueous solution. This has made these systems applicable in different areas of analytical chemistry, highlighting the increasing interest in their use in separation methods. The ability of micellar systems to solubilize hydrophobic compounds in aqueous solutions, to improve different analytical methodologies, or to develop new analytical methods due to the possibility of increasing sensitivity or selectivity should be emphasized.

In 1980, Armstrong and Henry showed the possibility of employing solutions of surfactants at a concentration above their c.m.c. as mobile phases for high performance liquid chromatography (HPLC), giving rise to micellar liquid chromatography (MLC). This technique, in which nonpolar chemically bonded stationary phases are generally used, constitutes an interesting alternative to aqueous–organic mobile phases in HPLC since micellar mobile phases are low cost and have low toxicity as compared with aqueous–organic mobile phases.

The great variety of interactions that are possible in MLC separations, for example electrostatic, hydrophobic and esteric, and the modification of stationary phase by adsorption of monomeric surfactants, make these systems more complicated than conventional reversed-phase HPLC (RP-HPLC) with aqueous–organic mobile phases. Micelles are not static species, but they exist above the c.m.c. in equilibrium with surfactant monomers. In a chromatographic column, surfactant monomers can be adsorbed on the surface of the stationary phase. For most surfactants and stationary phases, the amount of surfactant adsorbed remains constant after equilibrium between mobile and stationary phase is reached. The adsorption of a surfactant on a silica-bonded stationary phase, such as C_1, C_8 and C_{18}, can occur in two ways:

1. *Hydrophobic adsorption*. The alkyl hydrophobic chain of the surfactant is adsorbed and the ionic head is in contact with the polar solution, conferring on the stationary phase some ion-exchange properties when charged solutes are separated.
2. *Silanophilic adsorption*. The ionic head of the surfactant is adsorbed by the stationary phase, thus acquiring a more hydrophobic character.

Figure 1 shows the different equilibria existing in MLC. First, a solute can be partitioned between the aqueous mobile phase and the micellar mobile pseudophase, this equilibrium being controlled by a distribution coefficient P_{MW}. Second, this solute can be in equilibrium between the stationary phase and the micellar pseudophase, which is characterized by a distribution coefficient P_{SM}, and finally, a third equilibrium can be established for the solute distribution between stationary and aqueous mobile phases (P_{SW}).

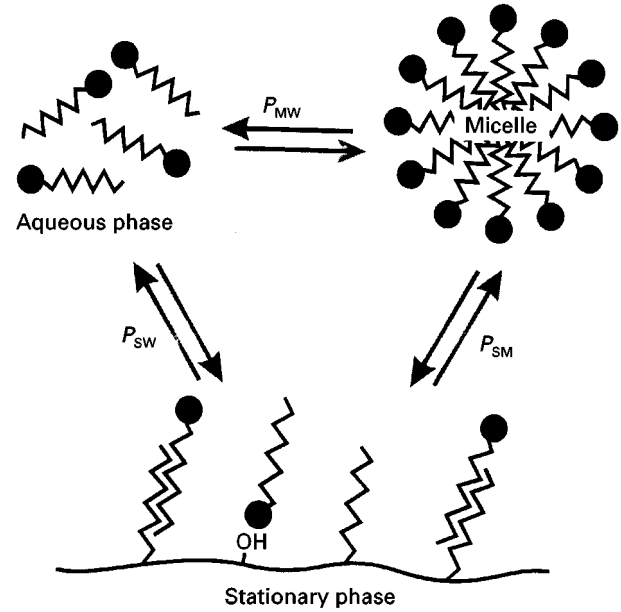

Figure 1 Distribution equilibria of a solute in micellar liquid chromatography. (Reproduced with permission from Marina ML and García MA (1997) *Journal of Chromatography A* 780: 103–116, copyright Elsevier Science Publishers B.V.)

Equations Describing Solute Retention in Micellar Liquid Chromatography

Table 1 groups some of the different models developed to describe solute retention in MLC. Some physicochemical models explain the variation of solute retention generally as a function of one or two experimental variables (micellar concentration, organic modifier concentration and pH). Empirical models, without a chemical sense, have also been developed to predict solute retention in MLC under different experimental conditions.

Purely Aqueous Micellar Systems

From equilibria taking place in MLC represented in Figure 1, equations have been developed relating chromatographic retention and concentration of micellized surfactant in solution.

Equation [1] (Table 1) relates the solute elution volume (V_e) in MLC with the micellized surfactant concentration in the mobile phase (C_M) (total surfactant concentration in solution minus c.m.c.). V_S, V_M and v are the stationary phase volume, the void volume of the column and the surfactant molar volume,

Table 1 Retention modelling in MLC

Model	*Equations*	
Physico-chemical	$V_S/(V_e - V_M) = \{\nu(P_{MW} - 1)/P_{SW}\}C_M + 1/P_{SW}$	[1]
	$1/k = \{K_2/\phi[L_S]K_1\}C_M + 1/\phi[L_S]K_1$	[2]
	$k = (V_S/V_M).(P_{SW}/\nu C_M)$	[3]
	$k = \dfrac{k_0(1 + K_2[C_M]) + k_1(1 + K_4[C_M])K_{am}/[H^+]}{1 + K_2[C_M] + (1 + K_4[C_M])K_{am}/[H^+]}$	[4]
	$k = \dfrac{\phi K_1[L_S](1 + K_4[A_M])}{1 + (K_3 + K_4)[A_M] + K_2[C_M](1 + K_3[A_M]) + K_3K_4[A_M]^2}$	[5]
Empirical relationships	$\ln k = -S\varphi + \ln k_0$	[6]
	$1/k = A\mu + B\varphi + C\mu\varphi + D$	[7]
	$1/k = A\mu + B\varphi^2 + C\varphi + D\mu\varphi + E$	[8]

respectively. If solute retention is expressed as the retention factor (k), a similar equation is obtained (eqn [2]) relating $1/k$ with C_M through the solute–micelle association constant per monomer, K_2. Here ϕ is the phase ratio (the ratio of the stationary phase volume to the volume of the mobile phase in the column, V_S/V_M), $[L_S]$ is the stationary phase concentration and K_1 is the binding constant for the solute between the bulk solvent and the stationary phase.

Equations [1] and [2] show that retention of a solute in MLC decreases when micelle concentration in the mobile phase increases. This is in contrast to reversed-phase ion-interaction (or ion-pairing) chromatography, where the surfactant concentration is below the c.m.c. (that is, no micelles exist), and the addition of an ionic surfactant increases retention for compounds that interact electrostatically with it.

For very hydrophobic compounds, a direct transfer retention mechanism from the micellar mobile phase to the modified stationary phase has been proposed. A limit theory has been developed for those compounds where the amount of the solute in the non-micellar aqueous mobile phase can be considered negligible. In this case, k is related to C_M through eqn [3] in Table 1.

For ionized solutes (weak acids, bases and zwitterionic solutes), some equations have also been developed relating k with C_M and pH. As an example, eqn [4] in Table 1 is the derived model for a weak acid. In this equation k_0 and k_1 are the limiting retention factors for the neutral and dissociated forms, respectively, K_4 is the association constant of the ionized form of the solute with the micellar phase, and K_{am} is the acid dissociation equilibrium constant. The variation of k with pH at a constant micellized surfactant concentration is sigmoidal. Since a shift in the ionization constants can be obtained when the micellized surfactant concentration is modified, optimization of separation conditions must be attained considering both variables simultaneously.

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Hybrid Micellar Systems

The addition of an organic modifier to a micellar solution can modify the characteristics of the micellar system (c.m.c. and the aggregation number). This can cause a variation of the solute–micelle interactions that, in turn, can change the chromatographic retention. On the one hand, a high concentration of alcohol can destroy the micellar structure, but on the other hand, the alcohol modifies the structure and composition of the stationary phase because it solvates the bonded hydrocarbon chain. Logically, the separation mechanism with the so-called hybrid mobile phases (micellar phases modified by alcohols) should be more similar to that for conventional aqueous–organic mobile phases than for purely aqueous micellar phases. However, if the integrity of the micelles remains, the addition of an alcohol to micellar mobile phases will not create an aqueous–organic system.

Both physicochemical and empirical models have been developed to describe the retention of solutes with hybrid mobile phases.

Physicochemical models Equation [5] (Table 1), which relates a solute retention factor with the micellized surfactant and alcohol concentrations, can be considered an extension of eqn [2]. This model considers the modification of stationary phase sites and micelle concentration due to the presence of an alcohol, that is, the alcohol can compete with the solute for interaction with the stationary phase and micelles. $[A_M]$ is the alcohol concentration in the mobile phase, and K_3 and K_4 are the association constants of the alcohol with the modified stationary phase and the micellar mobile phase, respectively. Based on the value of these constants and alcohol concentration, some simplified equations can be obtained. This model can predict a nonlinear, linear or quadratic variation of the retention factor with the alcohol concentration in the mobile phase (when micellized surfactant concentration is constant) and a linear variation of the inverse of retention factor with the micellized surfactant concentration (when alcohol concentration remains constant).

Empirical equations These models have no chemical background but are very valuable tools for predicting solute retention as a function of different variables. Among the different empirical equations reported in literature, models can be found relating solute retention to: (1) the organic modifier concentration, and (2) organic modifier and surfactant concentrations.

Empirical equations relating solute retention in MLC to organic modifier concentration Equation [6] (Table 1) is the simplest model relating the retention factor to the organic modifier concentration when surfactant concentration is constant. φ is the volume fraction of the organic modifier, S the eluent-strength parameter, and k_0 the retention factor in the absence of the organic modifier. Although this model can explain the decrease in solute retention observed in the presence of organic modifiers, deviations from linearity can be seen and some significant differences are obtained between the intercept and the experimental retention factor in the absence of an organic modifier. From an experimental viewpoint, its applicability is limited because the variation of the surfactant concentration is not considered.

Empirical equations relating solute retention in MLC to organic modifier and surfactant concentrations Equations have been obtained relating the logarithm of the retention factor to the volume fraction of the organic modifier (φ) and to the total surfactant concentration in the mobile phase (μ), but their applicability is limited. Other models have been proposed relating the inverse of the retention factor with these two variables and these, from which eqns [7] and [8] (Table 1) are examples, have shown a more general application range.

An extension of the iterative regression optimization strategy to multiparameter optimizations for the separation of ionic compounds in MLC has also been reported. The parameters examined are surfactant concentration, alcohol concentration and pH. Fairly regular (linear, weakly curved) retention behaviour of compounds as a function of the parameters results in an efficient optimization using a relatively small number of initial experiments.

All the models presented above require a mathematical equation, derived from chemical considerations, or are empirical in nature, but there are other methods that, although also empirical, do not have such requirements; these are artificial neural networks (ANNs). Although ANNs have been known for years, they have been applied only recently to model retention in MLC with hybrid eluents. ANNs are a very promising alternative to classical statistical methods for retention modelling studies in MLC.

Efficiency

One of the main drawbacks of MLC techniques is the loss observed in the chromatographic efficiency as compared with that obtained in RPLC with aqueous–organic mobile phases. This efficiency loss is attributed to the increase in the resistance of solute mass transfer from the mobile phase to the stationary phase. However, the addition of small quantities of an organic modifier to the mobile phase and an increase in the working temperature have shown that efficiencies similar to those obtained in RPLC with aqueous–organic mobile phases may be attained. Other suggestions include working with low flow rates and low surfactant concentrations. Indeed, it has been shown that the use of a high surfactant concentration in the mobile phase may cause efficiency loss.

Surfactant adsorption on the stationary phase seems to have a great influence on the efficiency. The addition of a short or medium chain alcohol causes surfactant desorption out of the stationary phase and improves efficiency. This effect increases with increase in the modifier's concentration and hydrophobicity.

Elution Strength of Micellar Mobile Phases

A disadvantage of MLC techniques is that the eluent strength of micellar mobile phases is quite small.

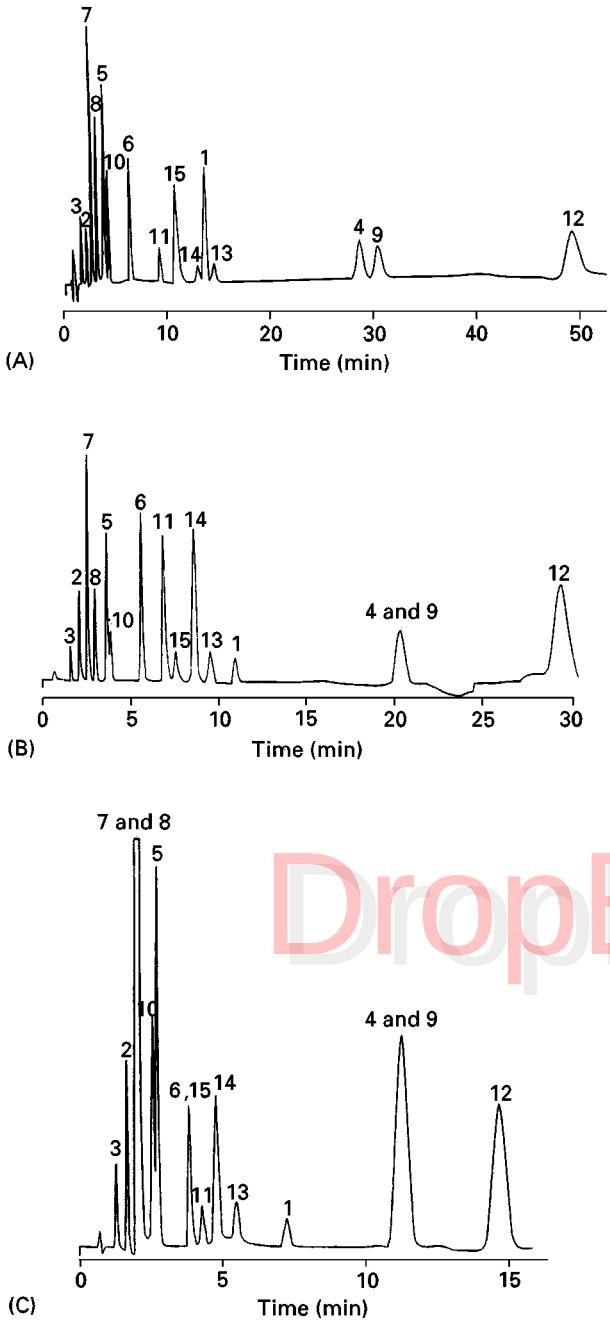

Figure 2 Chromatograms corresponding to the separation of a mixture of 15 benzene and naphthalene derivatives. (A) 0.02 mol L^{-1} SDS modified with 5% *n*-butanol. (B) 0.035 mol L^{-1} SDS modified with 5% *n*-butanol. (C) 0.035 mol L^{-1} SDS modified with 10% *n*-butanol. Key: 1, benzene; 2, benzylic alcohol; 3, benzamide; 4, toluene; 5, benzonitrile; 6, nitrobenzene; 7, phenol; 8, 2-phenylethanol; 9, chlorobenzene; 10, phenylacetonitrile; 11, 3,5-dimethylphenol; 12, naphthalene. Column: Hypersil C_{18} (10 cm × 4.0 mm i.d.). (Reproduced with permission from García MA, Vera S, Bombín M and Marina ML (1993) Optimization of the separation selectivity of a group of benzene and naphthalene derivatives in micellar high-performance liquid chromatography using a C_{18} column and alcohols as modifiers in mobile phase. *Journal of Chromatography* 646: 297–305, copyright Elsevier Science Publishers B.V.)

Although the eluent strength of purely micellar eluents increases when the micelle concentration in the mobile phase increases, an increase of the micelle concentration in the mobile phase generally causes an efficiency loss. For these reasons, the addition of organic modifiers to micellar mobile phases is of great interest since it is possible to increase both eluent strength and efficiency. As an example, **Figure 2** shows the chromatograms corresponding to the separation of a mixture of 15 benzene and naphthalene derivatives when the following sodium dodecyl sulfate (SDS) mobile phases are used: 0.02 mol L^{-1} SDS modified with 5% *n*-butanol (chromatogram A), 0.035 mol L^{-1} SDS modified with 5% *n*-butanol (chromatogram B) and 0.035 mol L^{-1} SDS modified with 10% *n*-butanol (chromatogram C). It can be observed that for the same alcohol percentage in the mobile phase, the elution strength increases with the surfactant concentration (Figures 2A and 2B), while for the same surfactant concentration in the mobile phase, elution strength increases with the alcohol percentage (Figures 2B and 2C).

The eluent strength of a micellar mobile phase modified by a short or medium chain alcohol such as methanol, propanol or butanol increases with the length of the alcohol chain as in conventional aqueous–organic systems.

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Separation Selectivity

The rate of change in retention of different solutes varies with charge and hydrophobicity of the solute as well as with the length of alkyl chain, charge and concentration of micelles. This causes inversions of elution order that are the result of two competing equilibria: the solute–micelle association, characterized by K_2, and the solute–stationary phase interaction, characterized by P_{SW}. The parameters K_2 and P_{SW} have a different effect on retention. When P_{SW} increases, retention also increases, but when K_2 increases, retention decreases. When the surfactant concentration in the mobile phase increases, the effect that K_2 has on retention also increases and reversals in elution order can be obtained if the difference in K_2 values for two solutes is large. Therefore, separation selectivity in MLC can be controlled by modifying surfactant nature and concentration. Furthermore, when organic modifiers are added to the mobile phase, the solvent strength parameter for a group of compounds does not have the same ranking for different alcohols owing to the different interaction of these modifiers with the micelles. For these reasons, MLC techniques are very interesting for chromatographic separation.

Although the conditions that optimize separation selectivity in MLC can vary with the nature of the solutes, several workers have shown an increase in separation selectivity for aromatic compounds in MLC with hybrid eluents when the micelle concentration in the mobile phase decreases. However, for a group of amino acids and peptides, an increase in micelle concentration can cause an increase or decrease in selectivity, or even an inversion of the peaks.

The effect of the organic modifier content in the mobile phase seems to be clearer. Generally, separation selectivity in MLC is improved in the presence of an organic modifier and increases with the volume fraction of the modifier in the mobile phase. This result is opposed to that observed in conventional RPLC with aqueous–organic mobile phases in which an increase of the organic modifier content causes a decrease of solute retention and selectivity. The selectivity enhancement observed in MLC when the solvent strength increases has been attributed to the competing partitioning equilibria in micellar systems and/or to the unique abilities of micelles to compartmentalize solutes and organic solvents. For some compounds, however, selectivity can decrease with the content of the alcohol in a micellar (SDS) mobile phase. In this case, for pairs of peaks where the selectivities are reduced by increasing alcohol concentration, a selectivity enhancement is observed with increasing micelle concentration and vice versa. Micelles and alcohols compete to interact with solutes affecting the role of one another in controlling retention and selectivity. The mutual effects of micelles and organic modifiers on each other also require a simultaneous optimization of these two parameters.

The retention mechanism of a solute in MLC can have implications for selectivity. If the retention of a solute in the chromatographic system takes place through a direct transfer mechanism, then the retention factor can be expressed by eqn [3] (Table 1). In this case, and if the surfactant concentration in the mobile phase is high, the selectivity coefficient (α) for a pair of solutes can be calculated from the ratio of their distribution coefficients between the stationary and micellar phases (P_{SM}):

$$\alpha = P_{SM1}/P_{SM2} \qquad [9]$$

This equation is useful for two reasons. First, because knowledge about the retention mechanism of compounds in the chromatographic system can be enhanced. In fact, if the experimental selectivity coefficient for a pair of solutes is constant and coincides with the ratio of their respective distribution coefficients, P_{SM}, it can then be assumed that retention occurs through a direct transfer from the micellar phase to the stationary phase. Second, calculation of the selectivity coefficient from eqn [9] enables prediction of the separation selectivity of two compounds in the chromatographic system, provided the distribution coefficients (P_{SM}) of the solutes are known.

As an example, **Figure 3** shows the variation of theoretical and experimental selectivity coefficients as a function of the micellized surfactant concentration in two mobile phases, SDS/5% *n*-propanol (Figures 3A, 3B and 3C) and hexadecyltrimethylammonium bromide (CTAB) modified by 5% *n*-butanol (Figures 3D, 3E and 3F) for three pairs of aromatic solutes. For pyrene/acenaphthene, both of which are highly hydrophobic, a direct transfer mechanism can be assumed for any surfactant concentration in these mobile phases. For pyrene/toluene a direct transfer mechanism can only be assumed for pyrene in all surfactant concentrations. For pyrene/benzamide, benzamide does not experience a direct transfer mechanism except at very high surfactant concentrations. Figure 3 shows that, when both solutes experience a direct transfer mechanism, the experimental and theoretical selectivity coefficients are very similar for all surfactant concentrations in solution, and it is therefore possible to predict the selectivity coefficient from the partition coefficients P_{SM} for the two solutes. When one of the two solutes does not experience a direct transfer mechanism, the theoretical and experimental selectivities are different. This difference decreases under conditions in which the direct transfer mechanism is favoured, i.e. by increasing the solute hydrophobicity, solute–micelle association constants, surfactant concentration in mobile phase and, for mobile phases modified by alcohols, by increasing the polarity of the alcohol. Consequently, the separation selectivity for a pair of solutes shows a tendency to match a limit value close to the ratio of stationary–micellar partition coefficients of two solutes. In this case, the separation selectivity cannot be experimentally modified through a change in the surfactant concentration in the mobile phase.

Applications

Determination of Solute–Micelle Association Constants and Distribution Coefficients

From eqn [1] in Table 1 it can be seen that a plot of the term $V_S/(V_e - V_M)$ versus C_M is linear and the term '$\nu(P_{MW} - 1)$' can be obtained from the slope : intercept ratio. According to Berezin's treatment the term '$\nu(P_{MW} - 1)$' is equal to the solute–micelle

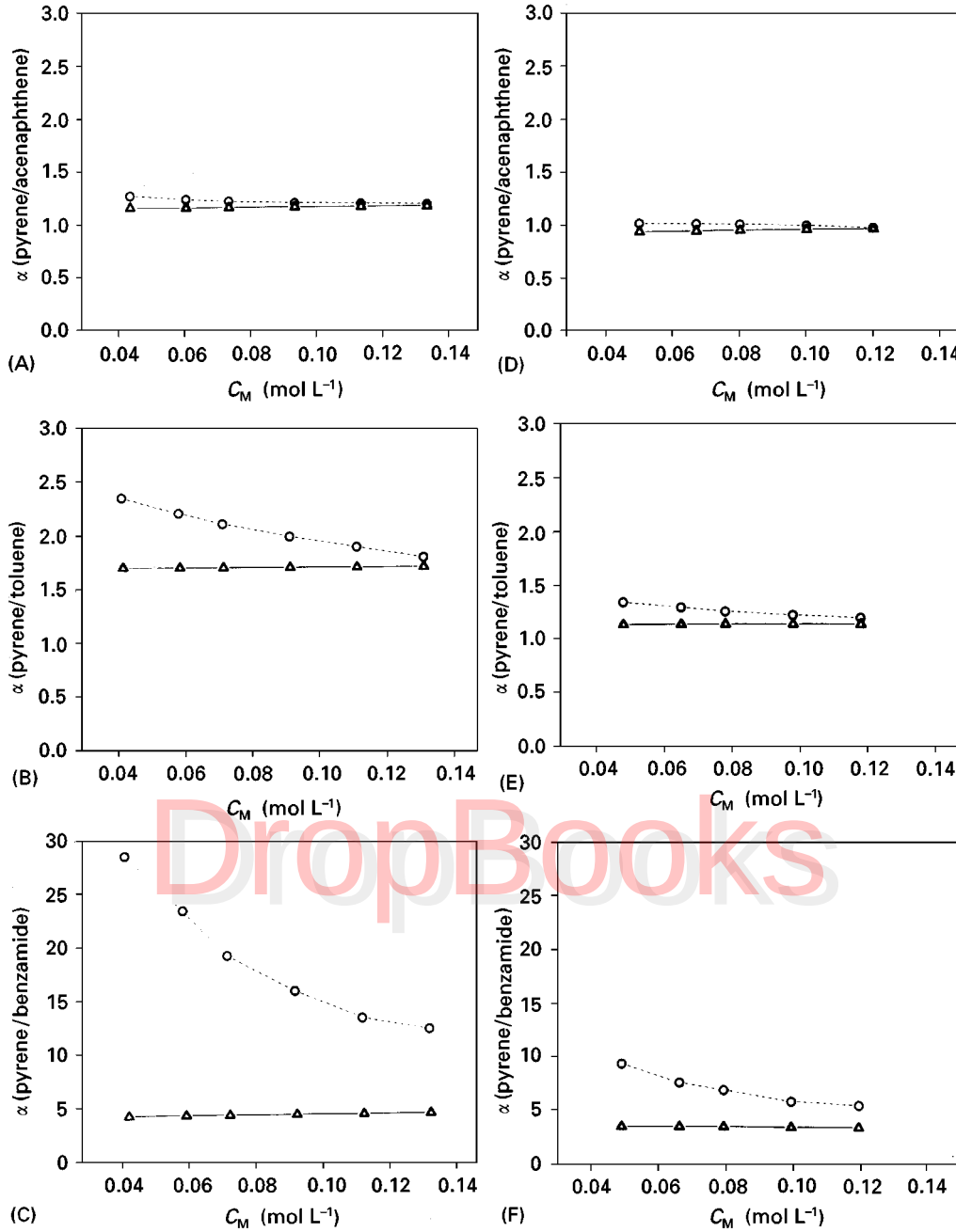

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Figure 3 Variation of the experimental (– –○– –) and theoretical (–△–) selectivity coefficients (α) as a function of the micellized surfactant concentration for three pairs of solutes: pyrene/acenaphthene (A and D), pyrene/toluene (B and E) and pyrene/benzamide (C and F). Mobile phases: A–C, SDS/5% *n*-propanol; D–F, CTAB/5% *n*-butanol. Column: Spherisorb C_8 (15 cm × 4.0 mm i.d.). (Reproduced with permission from García MA and Marina ML (1996) Influence of alcohol organic modifiers upon the association constants and retention mechanism for aromatic compounds in micellar liquid chromatography. *Journal of Liquid Chromatography and Related Technologies* 19: 1757–1776, copyright Marcel Dekker, Inc.)

association constant per monomer, K_2, which is the parameter most used to evaluate solute–micelle interactions. Also, the partition coefficient of the solute between bulk water and micelle, P_{MW}, can be obtained if the surfactant molar volume, v, is known. The distribution coefficient, P_{SW}, is obtained directly from the intercept and the distribution coefficient P_{SM} can be obtained from the ratio P_{SW}/P_{MW}.

In a similar way, the solute–micelle association constant per monomer, K_2, can be obtained directly from eqn [2] in Table 1 as the slope/intercept ratio of a straight line obtained from a plot of $1/k$ versus C_M.

If the solute–micelle association constant per monomer obtained from eqns [1] or [2] is multiplied by the aggregation number of the micelle, the association constant per micelle is obtained. On the other hand, P_{MW} and K_2 values only depend on the solute and the micellar system employed but not on the stationary phase.

Equations [1] and [2] have frequently been employed with the aim of determining solute–micelle association constants in pure and modified micellar media. Furthermore, good agreement has been found between the values of the association constants obtained by MLC and other techniques.

Figure 4 provides an example of the good linearity obtained for the variation of the term $V_S/(V_e - V_M)$ as a function of C_M for a group of 12 polycyclic aromatic hydrocarbons when an SDS micellar mobile phase modified by 5% *n*-butanol is used.

For highly hydrophobic solutes, the retention of which is described by eqn [3] in Table 1, the variation of $1/k$ as a function of C_M should give a straight line with an intercept equal to zero; the slope of the line should allow calculation of the distribution coefficient P_{SM}. In these cases, calculation of solute–micelle association constants is not possible and has no chemical meaning.

One of the main drawbacks that this method has is the intrinsic error derived from the determination of a magnitude from a quotient. Error obtained during the determination of K_2 increases with solute hydrophobicity since P_{SW} values for these compounds are elevated (intercept very small, see eqn [1]). With hybrid eluents, the value of P_{SW} decreases and the error in the determination of the solute–micelle association constants for very hydrophobic compounds also decreases (the intercept in eqn [1] increases).

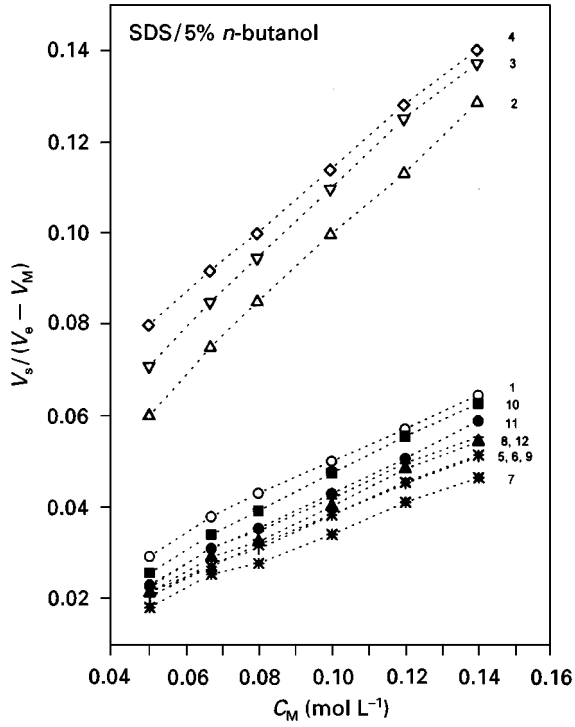

Figure 4 Variation of the term $V_s/(V_e - V_m)$ as a function of C_M for a group of 12 polycyclic aromatic hydrocarbons in an SDS micellar system modified by 5% *n*-butanol. Key: 1, naphthalene; 2, 1-naphthol; 3, 2-naphthol; 4, 1-naphthylamine; 5, pyrene; 6, phenanthrene; 7, 2,3-benzofluorene; 8, fluorene; 9, fluoranthene; 10, acenaphthylene, 11, acenaphthene; and 12, anthracene. Column: Spherisorb C_8 (15 cm × 4.0 mm i.d.). (Reproduced with permission from Marina ML and García MA (1997) *Journal of Chromatography A* 780: 103–116, copyright Elsevier Science Publishers B.V.)

Rapid Elution Gradients

Gradient elution techniques are the most versatile and popular techniques for solving the general elution problem in liquid chromatography. The advantages of gradient elution are enhanced peak resolution, faster analysis times, and better detectability. The major disadvantage is that the compositions of the stationary and mobile phases change during the course of the separation and column regeneration is needed before the next analysis. In order to perform gradient elution in MLC, the concentration of micelles and/or organic modifier may be increased during the course of the separation. In a micellar concentration gradient, re-equilibration time at the end of a gradient run is not necessary, and in an organic modifier gradient the re-equilibration time is very short.

Micellar gradients Rapid micellar elution gradients can be performed in MLC because re-equilibration time for the column is not necessary. This is because the amount of surfactant adsorbed on the stationary phase remains practically constant after reaching equilibrium when the concentration of surfactant in the mobile phase is above the c.m.c. Accordingly, micellar elution gradients are compatible with electrochemical detection. **Figure 5** shows the separation of eight organic compounds using a micellar gradient and electrochemical detection.

Organic modifier gradients Organic solvent gradients in MLC require short re-equilibration times at the end of the gradient mainly due to the small range of organic modifier concentration used in MLC in order to maintain micelle integrity. In this case, the change in the concentration of organic modifier is not sufficient to change the concentration of adsorbed surfactant monomer in the stationary phase. In MLC,

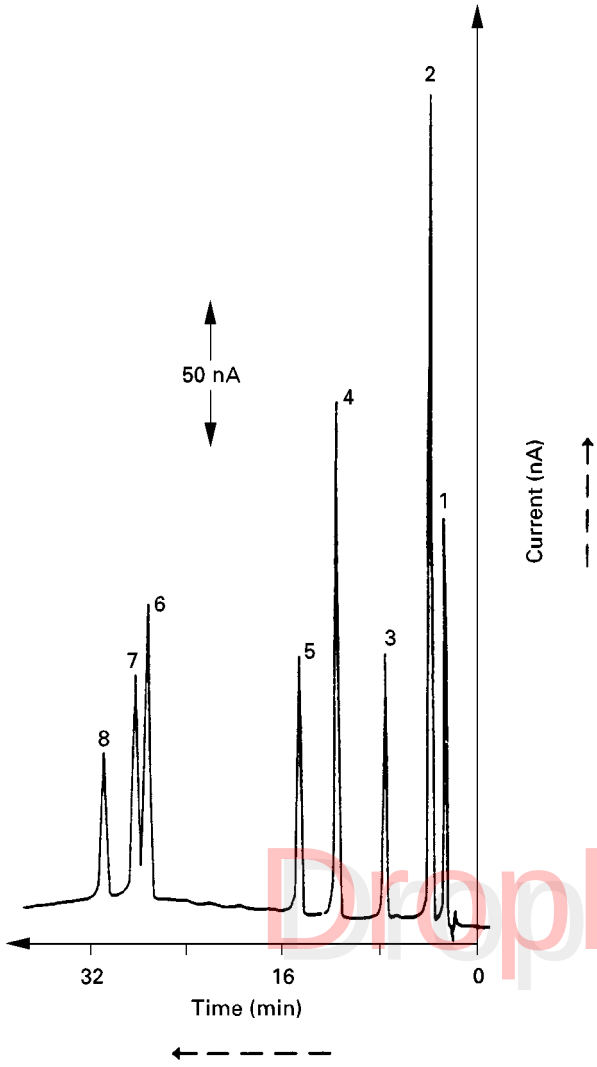

Figure 5 Micellar gradient elution separation with electrochemical detection at + 1.2 V. Flow rate: 1.0 mL min^{-1}. Mobile phase A: 0.05 mol L^{-1} SDS/3% 1-propanol, pH 2.5 with phosphate buffer, sodium perchlorate added to balance conductivity with solvent B. Mobile phase B: 0.112 mol L^{-1} SDS/3% 1-propanol, pH 2.5 with phosphate buffer. Gradient program A to B in 12 min. Key: 1, hydroquinone; 2, resorcinol; 3, catechol; 4, phenol; 5, *p*-nitrophenol; 6, *o*-nitrophenol; 7, *p*-chlorophenol; 8, *p*-bromophenol. Column: Altex Ultrasphere C_{18} (15 cm × 4.6 mm i.d.). (Reproduced with permission from Dorsey JG, Khaledi MG, Landy JS and Lin JL (1984) Gradient elution micellar liquid chromatography. *Journal of Chromatography* 316: 183–191, copyright Elsevier Science Publishers B.V.)

organic modifier gradients are useful since although a limited range of organic modifier may be used, the solvent strength can be compensated with a concurrent micelle concentration gradient. **Figure 6** shows the separation of a mixture of amino acids and peptides in MLC under isocratic and gradient conditions.

Enhancement of Detection Sensitivity

Luminescence detection can be improved in MLC because many solutes show enhanced fluorescence and in some cases room temperature liquid phosphorescence when associated with micelles. The fluorescence intensity of certain compounds in micellar media can be drastically increased as a result of solubilization in the micelle. The location of a solute in the anisotropic medium of micelles, which have a large microenvironment viscosity and different polarity from the aqueous bulk solvent, would result in a decrease in the freedom of movement, shielding of the compounds from nonradiation deactivation, and/or an increase in quantum efficiency. This leads to intensified fluorescence signals and thus to better sensitivity and lower detection limits. Room temperature phosphorescence in solution is possible in the presence of ionic micelles and heavy atom counterions, which increase the population of the triplet excited state molecules and protect them from radiationless deactivation.

Furthermore, many metal–dye complexes show increased absorbance in the presence of micelles. This is

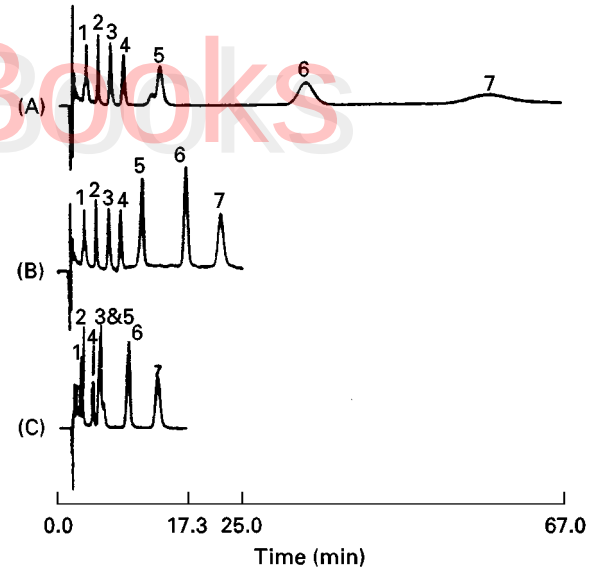

Figure 6 Separation of a seven-component test mixture. Mobile phase: 0.30 mol L^{-1} SDS, 0.02 mol L^{-1} phosphate buffer, pH 2.5 with propanol added. (A) Isocratic separation with 3% 2-propanol, (B) gradient separation with 3 to 15% 2-propanol, and (C) isocratic separation with 15% 2-propanol. Key: 1, aspartic acid–phenylalanine; 2, phenylalanine; 3, lysine–phenylalanine; 4, phenylalanine–phenylalanine; 5, triphenylalanine; 6, tetraphenylalanine; and 7, pentaphenylalanine. Column: Nucleosil C_{18} (15 cm × 4.6 mm i.d.). (Reproduced with permission from Madamba-Tan LS, Strasters JK and Khaledi MG (1994) Gradient elution in micellar liquid chromatography II. Organic modifier gradients. *Journal of Chromatography A* 683: 335–345, copyright Elsevier Science Publishers B.V.)

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due to the capacity of the micelles to produce hyperchromic and bathochromic displacements. Generally, these displacements result in greater sensitivity. In UV/Vis spectrophotometry, the upward displacement of the λ_{max} of the complex, together with the effect that micellar solutions also have on the λ_{max} of the ligand, normally enable a more sensitive metal ion determination than that possible in nonmicellar media.

Figure 7 shows a comparison of the detected fluorescent peaks of identical concentrations of three aromatic solutes separated by HPLC. The enhanced fluorescence obtained with an SDS mobile phase with respect to that obtained with a methanol/water mobile phase is observed.

Direct Injection of Biological Fluids

From a bioanalytical viewpoint, a very useful application of MLC is the ability to inject biological fluids (serum, plasma and urine) directly into a chromatographic system with no protein precipitation, analyte extraction steps or pressure build-up problems. These advantages are extremely beneficial in areas such as therapeutic drug monitoring because the analyte extraction steps, traditionally necessary in chromatographic methods, are eliminated. In this way, analysis time is reduced and accuracy and precision are increased because the possible analyte co-precipitation with the protein is avoided.

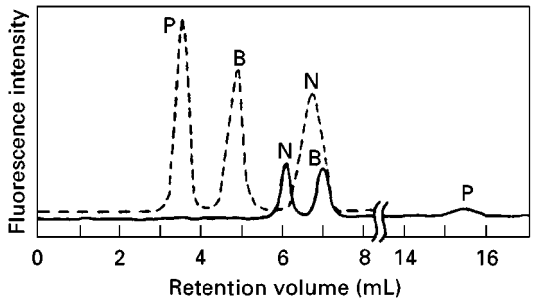

Figure 7 Comparison of the detected fluorescent peaks of identical concentrations of pyrene (P), biphenyl (B) and naphthalene (N) separated by HPLC on a 30 cm × 4.0 mm i.d. alkylnitrile column. The broken line (– – –) shows the separation and enhanced fluorescence obtained with the 0.024 mol L^{-1} SDS mobile phase. The solid line (——) shows an analogous separation done with a traditional 40 : 60 methanol/water mobile phase. In both separations 10 μL of solution containing 1.3×10^{-7} g (N), 7.0×10^{-8} g (B) and 1.1×10^{-8} g (P) were injected. (Reproduced with permission from Armstrong DW, Hinze WL, Bui KH and Singh HN (1981) Enhanced fluorescence and room temperature liquid phosphorescence detection in pseudophase liquid chromatography (PLC). *Analytical Letters* 14: 1659–1667, copyright Marcel Dekker, Inc.)

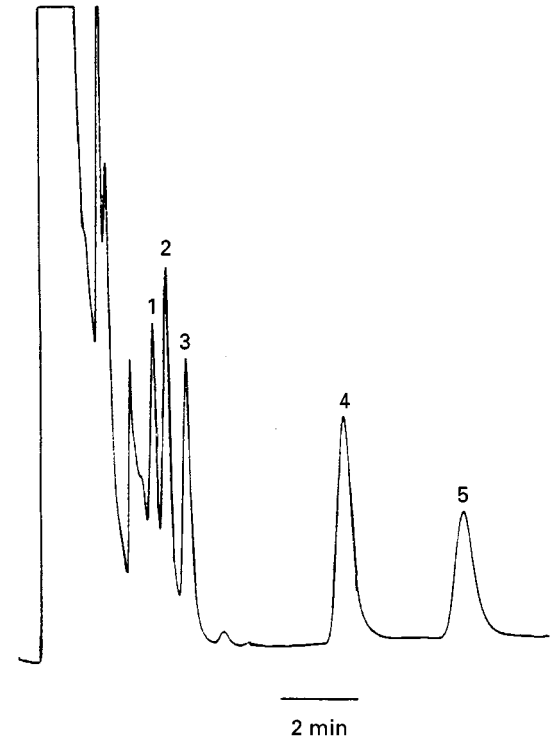

Figure 8 Chromatogram of a urine sample spiked with: 1, amiloride (30 μg mL^{-1}, 3.67 min); 2, spirolactone (5 μg mL^{-1}, 4.02 min); 3 metandienone (1.2 μg mL^{-1}, 4.57 min); 4, phenylpropanolamine (56 μg mL^{-1}, 8.63 min); and 5, clostebol (30 μg mL^{-1}, 11.67 min). Mobile phase: 0.1 mol L^{-1} SDS/3% 1-pentanol. Flow rate: 1 mL min^{-1}. Column temperature: 60°C. UV detection at 260 nm. Column: Spheri-5 C_{18} (10 cm × 4.6 mm i.d.). (Reproduced with permission from Carretero I, Maldonado M, Laserna JJ, Bonet E and Ramis-Ramos G (1992) Detection of banned drugs in sport by micellar liquid chromatography. *Analytica Chimica Acta* 259: 203–210, copyright Elsevier Science Publishers, B.V.)

Micellar systems such as SDS or polyoxyethylene lauryl ether (Brij-35) solubilize the serum proteins and cause their elution with the void volume. Furthermore, surfactant monomers compete with the analyte for protein-binding sites, thereby releasing it for complete quantitation.

Figure 8 shows the separation by MLC of a mixture containing diuretics (amiloride and spirolactone), anabolic steroids (metandienone and clostebol) and a stimulant (phenylpropanolamine) added to a urine sample at concentrations of μg mL^{-1}.

Hydrophobicity Estimation for Organic Compounds

Another interesting possibility of MLC techniques is their application to the quantitation of physicochemical properties of biologically active compounds in QSAR (quantitative structure–activity

relationships) studies, especially for the prediction of hydrophobicity.

Hydrophobicity is commonly understood as a measure of the relative tendency of a solute to prefer a nonaqueous rather than an aqueous environment. Biological activity of many compounds, bioaccumulation of organic pollutants, and soil sorption of contaminants have all been correlated to the lipophilic character of the molecules concerned.

The quantitation of hydrophobicity has both diagnostic and predictive value in various disciplines such as drug design, toxicology and environmental monitoring.

Traditionally, the logarithm of the octanol–water partition coefficient ($\log P_{OW}$) of the nonionized form of a solute has been the most common parameter used to measure the hydrophobicity. The standard 'shake-flask' method for determining partition coefficients in liquid–liquid systems has several serious disadvantages. This fact, together with the use of a bulk solvent such as octanol as a model for complex systems such as biomembranes, has been occasionally criticized and has instigated the search for other indirect methods for evaluating hydrophobicity. Among these methods, chromatographic techniques such as reversed-phase TLC and HPLC can be highlighted. From 1977 several QSRR (quantitative structure–retention relationships) studies have appeared in the literature relating the biological activity of a solute and its retention in a chromatographic system. Good linear relationships between the logarithm of the retention factor ($\log k$) for series of organic compounds determined by RPLC and their $\log P_{OW}$ have been obtained.

The linear relationships obtained between $\log k$ determined by chromatographic techniques and $\log P_{OW}$ are based on the relationship existing between the logarithms of the distribution coefficients of a solute in two different systems, provided the interactions that the solute experiences in these systems are similar and the relationship can be expressed by an equation of the Collander type ($\log P_1 = a_1 \log P_2 + a_2$, where P_1 and P_2 are the distribution coefficients of the solute in the two different phases and a_1 and a_2 are constants).

The good linear correlations obtained between $\log k$ and $\log P_{OW}$ in RP-HPLC suggest that the Collander relationship is satisfied, that is, that the interactions of a solute in an aqueous–stationary phase system are similar to the interactions that the solute experiences in an aqueous–octanol one.

As micelles are considered to be simple chemical models for biomembranes, MLC has been investigated as an interesting possibility for evaluating the hydrophobicity of organic compounds. *n*-Octanol is an isotropic solvent in which the molecular size and shape of the molecules are not important factors; however, micellar systems, like biomembranes, have amphiphilic properties and are anisotropic media so that the size and shape of molecules influence their penetration through them. The solubilization (or partitioning of solute into micelles) closely resembles that of lipid bilayers and both of these are different from the two-phase octanol–water system.

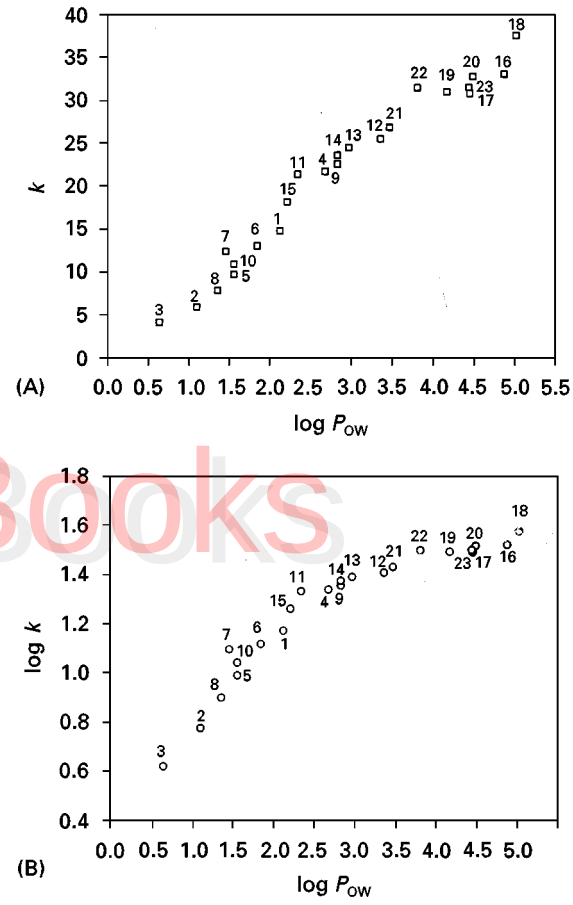

Figure 9 Variation of k (A) and $\log k$ (B) with $\log P_{OW}$ for a group of 23 benzene derivatives and polycyclic aromatic hydrocarbons in a 0.05 mol L^{-1} CTAB/3% *n*-propanol mobile phase. Key: 1, benzene; 2, benzylic alcohol; 3, benzamide; 4, toluene; 5, benzonitrile; 6, nitrobenzene; 7, phenol; 8, 2-phenylethanol; 9, chlorobenzene; 10, phenylacetonitrile; 11, 3,5-dimethylphenol; 12, naphthalene; 13, 1-naphthol; 14, 2-naphthol; 15, 1-naphthylamine; 16, pyrene; 17, phenanthrene; 18, 2,3-benzofluorene; 19, fluorene; 20, fluoranthene; 21, acenaphthylene; 22, acenaphthene; and 23, anthracene. Column: Spherisorb C_8 (15 cm × 4.0 mm i.d.). (Reproduced with permission from García MA and Marina ML (1994) Study of the k' or $\log k' - \log P_{OW}$ correlation for a group of benzene derivatives and polycyclic aromatic hydrocarbons in micellar liquid chromatography with a C_8 column. *Journal of Chromatography A* 687: 233–239, copyright Elsevier Science Publishers B.V.)

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Contradictory results have been obtained concerning which of the two parameters (k or $\log k$) best correlates with $\log P_{OW}$ in MLC. **Figure 9** shows the variation of k (Figure 9A) and $\log k$ (Figure 9B) with $\log P_{OW}$ for a micellar mobile phase, 0.05 mol L^{-1} CTAB modified by 3% *n*-propanol, for a group of 23 benzene derivatives and polycyclic aromatic hydrocarbons. This figure shows that a good linear correlation between $\log k$ and $\log P_{OW}$ can be obtained for solutes with a low hydrophobicity, while, when high $\log P_{OW}$ values are attained, there exists a $\log P_{OW}$ value from which no further change for $\log k$ with $\log P_{OW}$ is obtained. This is due to the change in the retention mechanism of compounds from a three-equilibria mechanism to a direct-transfer mechanism from the micellar mobile phase to the stationary phase with increasing $\log P_{OW}$ for solutes. In fact, for highly hydrophobic compounds, which can become insoluble in water, the predominant equilibrium is the distribution between the micellar and stationary phases. Since these two phases are chemically similar, the distribution coefficient is close to unity and may become independent of solute hydrophobicity. In this way the variation of $\log k$ with $\log P_{OW}$ is represented by a curve.

Conclusion

MLC is a mode of HPLC in which solutions of surfactants at a concentration above their critical micellar concentration are employed as mobile phases. Three different equilibria exist for a solute in MLC. It can distribute between the aqueous mobile phase and the micellar mobile pseudophase, between the stationary phase and the micellar pseudophase, and between stationary and aqueous mobile phases. However, for highly hydrophobic compounds, a direct-transfer mechanism from the micellar to the stationary phase has been proposed. Solute retention is related to the concentration of micellized surfactant in the mobile phase through solute–micelle association constants or distribution coefficients that can be calculated as a direct application of MLC techniques.

The great number of interactions that are possible in MLC separations, such as electrostatic, hydrophobic and esteric, and the modification of the stationary phase by adsorption of monomeric surfactants, make these systems more complicated than conventional RP-HPLC. However, the fact that the amount of the surfactant adsorbed remains constant after equilibrium between mobile and stationary phases allows MLC techniques to achieve rapid micellar and organic modifier gradients.

The control of separation selectivity in MLC can be performed through a great number of parameters; these include the nature and concentration of the surfactant in the mobile phase, the presence of additives as organic modifiers and salts, and the pH. The fact that the addition of an organic modifier to micellar mobile phases can increase selectivity and reduce analysis time has increased the use of hybrid micellar mobile phases, which also preclude the efficiency loss inherent to MLC as compared to conventional RP-HPLC.

Other applications that can be cited in MLC techniques are directly derived from the special characteristics of micellar solutions. The sensitivity of the detection can be enhanced and biological fluids can be directly injected into the chromatographic systems because of the solubilization of the protein by some surfactants. Finally, the fact that micelles can be considered as chemical models for biomembranes has enabled the application of MLC to hydrophobicity estimation of organic compounds.

See also: **II/Chromatography: Liquid:** Mechanisms: Reversed Phases. **Electrophoresis:** Micellar Electrokinetic Chromatography. **III/Surfactants:** Liquid Chromatography.

Further Reading

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